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Frank C. Eisenschenk, Ph.D., Patent Attorney

REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 CFR 1.322
Docket No. C.R.115

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Stephen Noel Fitzgerald, Richard Joseph Fagan, Christine Power, Melanie Yorke, Jadwiga Bienkowska
Issued	:	April 20, 2010
Patent No.	:	7,700,724
Conf. No.	:	5399
For	:	Isolated INSP 163 Protein

Mail Stop Certificate of Corrections Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION
UNDER 37 CFR 1.322 (OFFICE MISTAKE)

Sir:

A Certificate of Correction for the above-identified patent has been prepared and is attached hereto.

In the left-hand column below is the column and line number where errors occurred in the patent. In the right-hand column is the page and line number in the application where the correct information appears.

Patent Reads:	**Application Reads:**
Column 2, line 32:	Page 2, line 31:
"MRNA"	--mRNA--

Column 5, line 47:	Page 7, line 22:
"β-amayloid"	--β-amyloid--
Column 10, lines 4-5:	Page 13, line 31:
"osteoarritis"	--osteoarthritis--
Column 13, line 36:	Page 18, line 27:
"comeal dystrophy"	--corneal dystrophy--
Column 13, line 43:	Page 18, line 32:
"diffuise large cell"	--diffuse large cell--
Column 13, line 50:	Page 19, line 3:
"athritis"	--arthritis--
Column 18, line 17:	Page 25, line 19:
"used herin"	--used herein--
Column 19, line 43:	Page 27, line 19:
"singie"	--single--
Column 20, line 46:	Page 29, line 4:
"Jones et aL,"	--Jones et al.,--
Column 20, line 48:	Page 29, line 5:
"Queen et aL,"	--Queen et al.,--
Column 21, line 31:	Page 30, line 8:
"MRNA"	--mRNA--
Column 21, line 32:	Page 30, line 8:
"CDNA"	--cDNA--

Column 22, line 11:	Page 31, line 8:
"MRNA"	--mRNA--
Column 23, line 33:	Page 33, line 4:
"15 nM"	--15mM--
Column 25, line 34:	Page 35, line 31:
"Johns Hopldns"	--Johns Hopkins--
Column 26, line 2:	Page 36, line 22:
"MRNA"	--mRNA--
Column 29, line 29:	Page 41, line 21:
"MRNA"	--mRNA--
Column 32, line 34:	Page 46, line 6:
"MRNA"	--mRNA--
Column 44, line 22:	Page 62, line 26:
"or SuperScript II"	--or SuperScript III--
Column 44, line 46:	Page 63, line 16:
"4 µl 25 nM"	--4 µl 25 mM--
Column 45, line 17:	Page 64, line 10:
"2 nM"	--2mM--
Column 48, line 62:	Page 69, line 11:
"(or 10 nM"	--(or 10mM--
Column 50, line 11:	Page 71, line 3:
"600 nM"	--600mM--

Column 50, line 32:	Page 71, line 16:
"15 nM"	--15mM--
Column 50, line 45:	Page 71, line 25:
"2.7 nM KCl; 1.5 nM KH_2PO_4; 8 nM"	--2.7mM KCl; 1.5mM KH_2PO_4; 8mM--
Column 50, lines 47-48:	Page 71, line 27:
"137 nM NaCl; 2.7 nM KCl; 1.5 nM"	--137mM NaCl; 2.7mM KCl; 1.5mM--
Column 50, lines 63-64:	Page 72, line 7:
"137 nM NaCl; 2.7 nM KCl; 1.5 nM KH_2PO_4; 8 nM"	--137mM NaCl; 2.7mM KCl; 1.5mM KH_2PO_4; 8 mM--.

A true and correct copy of pages 2, 7, 13, 18, 19, 25, 27, 29, 30, 31, 33, 35, 36, 41, 46, 62-64, 69, 71 and 72 of the specification as filed which support Applicants' assertion of the errors on the part of the Patent Office accompanies this Certificate of Correction.

Approval of the Certificate of Correction is respectfully requested.

Respectfully submitted,

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Attachments: Copy of pages 2, 7, 13, 18, 19, 25, 27, 29, 30, 31, 33, 35, 36, 41, 46, 62-64, 69, 71 and 72 of the specification
Certificate of Correction

affect the transport of polypeptide chains from the cytoplasm to a membrane bound compartment such as a secretory vesicle. Polypeptides that are targeted to the secretory vesicles are either secreted into the extracellular matrix or are retained in the plasma membrane. The polypeptides that are retained in the plasma membrane will have one or more transmembrane domains. Examples of secreted proteins that play a central role in the functioning of a cell are cytokines, hormones, extracellular matrix proteins (adhesion molecules), proteases, and growth and differentiation factors. Description of some of the properties of these proteins follows.

Cytokines

Cytokines are a family of growth factors secreted primarily from leukocytes, and are messenger proteins that act as potent regulators capable of effecting cellular processes at sub-nanomolar concentrations. Interleukins, neurotrophins, growth factors, interferons and chemokines all define cytokine families that work in conjunction with cellular receptors to regulate cell proliferation and differentiation. Their size allows cytokines to be quickly transported around the body and degraded when required. Their role in controlling a wide range of cellular functions, especially the immune response and cell growth, has been revealed by extensive research over the last twenty years (Boppana, S.B (1996) Indian. J. Pediatr. 63(4):447-52). Cytokines, as for other growth factors, are differentiated from classical hormones by the fact that they are produced by a number of different cell types rather than just one specific tissue or gland, and also affect a broad range of cells via interaction with specific high affinity receptors located on target cells.

All cytokine communication systems show both pleiotropy (one messenger producing multiple effects) and redundancy (each effect is produced by more than one messenger) (Tringali, G. *et al.*, (2000) Therapie. 55(1):171-5; Tessarollo, L. (1998) Cytokine Growth Factor Rev. 9(2):125-137). An individual cytokine's effects on a cell can also be dependent on its concentration, the concentration of other cytokines, the temporal sequence of cytokines, and the internal state of the cell (cell cycle, presence of neighbouring cells, cancerous).

Although cytokines are typically small proteins (under 200 amino acids) they are often formed from larger precursors which are post-translationally spliced. This, in addition to mRNA alternative splicing pathways, give a wide spectrum of variants of each cytokine, each of which may differ substantially in biological effect. Membrane and extracellular

- SAP, which results in complement activation.
- PTX3, which mediates complement activation on apoptotic cells.
- Decorin, which modulates the classical pathway activation in the tissue.
- Gram negative bacteria proteins via lipid A, LPS and porins. OmpK36 (from *Klebsiella pneumoniae*) competes directly with IgG for binding to c1q.
- Viral proteins (enveloped and non-enveloped) like envelope protein gp41 of HIV-1, gp21 of HTLV-I, p15e of MuLV). The c1q domain binding to viruses might result in virus neutralization. C1q-gp41 interaction leads to enhanced infection of complement-receptor-bearing cells, instead of viral lysis. Interaction between HTLV-I peptide and the c1q domain might affect the fusion process required for syncytium formation.
- Pentraxins on apoptotic cells. C1q deficiency can cause SLE as a result of an impaired clearance of apoptotic cells. Surface blebs of apoptotic keratinocytes and peripheral blood mononuclear cells, which contain autoantigens are targeted in SLE. In c1q knockout mice, which have glomerulonephritis with immune deposits, a large number of apoptotic bodies are also present in diseased glomeruli. C1q might protect against autoimmunity by serving as an opsonin in the efficient recognition and physiological clearance of apoptotic cells, hence be required to maintain immune tolerance.
- β-amyloid and familial dementia peptides (to the N-terminal region). Classical pathway activation leads to inflammation in neuritic plaques.
- Cardiolipin and other anionic PLs, suggesting a possible role in the clearance of apoptotic and necrotic cells.

The C-terminal globular domain of the c1q subcomponents and collagen types VIII and X is important both for the correct folding and alignment of the triple helix and for protein-protein recognition events. For collagen type X it has been suggested that the domain is important for initiation and maintenance of the correct assembly of the protein (Kwan *et al.* 1991 J. Cell Biol.. 114:597-604). In adiponectin, the c1q domain can ameliorate

cause of spondylometaphyseal dysplasia japanese type (SMD). SMD comprises a heterogeneous group of heritable skeletal dysplasias characterized by modifications of the vertebral bodies of the spine and metaphyses of the tubular bones. Adiponectin (ACDC gene) is an important negative regulator in hematopoiesis and immune systems. It may be involved in ending inflammatory responses through its inhibitory functions. It inhibits endothelial NF-kappa-B signaling through a cAMP-dependent pathway as well as TNF-alpha-induced expression of endothelial adhesion molecules. Adiponectin is involved in the control of fat metabolism and insulin sensitivity. It is synthesized exclusively by adipocytes and secreted into plasma. Defects in ACDC are the cause of adiponectin deficiency. The result is a very low concentration of plasma adiponectin. Decreased adiponectin plasma levels are associated with obesity insulin resistance, and diabetes type 2. CORS-26 might be involved in arthritis, bone or skeletal disease, osteosarcoma, chondroblastoma and giant cell tumor (Schaffler *et al.* 2003 Biochim Biophys Acta. 1628(1):64-70. 2003 Biochim Biophys Acta. 1630(2-3):123-9). Mutation in the c1q domain of Complement c1q tumor necrosis factor-related protein 5 could lead to late-onset retinal degeneration (L-ORD), age-related macular degeneration (AMD) and/or blindness (Hayward *et al.* 2003 Hum Mol Genet. 12(20):2657-67).

BAFF and the apoptosis ligand APRIL (also named TALL-2, TRDL-1 and TNFSF-13), including EDA and TWEAK, belong to a subgroup of the THD family. This subfamily share functional properties such as cell survival and differentiation, and structural features such as the presence of a furin convertase cleavage site in the stalk region of the protein and a disulfide bond link between the E and F strands within the molecules (Mackay and Ambrose, 2003 Cytokine Growth Factor Rev. 14(3-4):311-24). Soluble BAFF has been detected in serum (furin cleavage site RNKR↓) and APRIL is predominantly secreted as a soluble molecule (furin cleavage site RKRR↓). Cleavage sites have also been detected in INSP163. BAFF has been implicated in B cell survival, maturation and activation (involved in B cell immune responses), in T cell activation, and in maintenance of Ig-secreting cells, suggesting a critical role in promoting humoral responses and the maintenance of immune tolerance. BAFF has a role in:

1. Autoimmune diseases and inflammation: BAFF has been implicated in rheumatoid arthritis (RA), osteoarthritis, Systemic lupus erythematosus (SLE), Sjögren syndrome, and multiple sclerosis (Thangarajh *et al.* 2004 J Neuroimmunol. 152(1-2):183-90).

conditions; particularly immune disorders, such as autoimmune disease, rheumatoid arthritis, osteoarthritis, psoriasis, systemic lupus erythematosus, and multiple sclerosis, inflammatory disorders, such as allergy, rhinitis, conjunctivitis, glomerulonephritis, uveitis, Crohn's disease, ulcerative colitis, inflammatory bowel disease, pancreatitis, digestive system inflammation, sepsis, endotoxic shock, septic shock, cachexia, myalgia, ankylosing spondylitis, myasthenia gravis, post-viral fatigue syndrome, pulmonary disease, respiratory distress syndrome, asthma, chronic-obstructive pulmonary disease, airway inflammation, wound healing, endometriosis, dermatological disease, Behcet's disease, neoplastic disorders, such as melanoma, sarcoma, renal tumour, colon tumour, haematological disease, myeloproliferative disorder, diseases associated with the disregulation of apoptosis, Hodgkin's disease, osteoporosis, obesity, diabetes, gout, cardiovascular disorders, reperfusion injury, atherosclerosis, ischaemic heart disease, cardiac failure, stroke, liver disease, AIDS, AIDS related complex, neurological disorders, male infertility, ageing and infections, including plasmodium infection, bacterial infection and viral infection, hereditary diseases, including hyper IgM syndrome (HIM, CD40L), type I autoimmune lymphoproliferative syndrome (ALPS, Fas/FasL), TNF-R1-associated periodic fever syndrome (TRAPS, TNF-R1), hypohidrotic ectodermal dysplasia (HED, EDA/EDAR), familial expansile osteolysis (FEO, RANK) and other pathological conditions. Preferably the disease is selected from autoimmune diseases, autoimmune inner ear disease, Labyrinthitis, Ménière disease and Ménière syndrome, Perilymphatic or labyrinthine fistula, Tinnitus, neurodegenerative diseases, amyloidosis, Alzheimer's disease, Parkinson's disease, familial dementia, inflammation (joint pain, swelling, anemia, or septic shock), infectious diseases, parasitic diseases, microbial diseases, bacterial diseases, viral diseases (HIV, HTLV, MuLV, *Streptococcus pneumoniae* and *Ascaris lumbricoides* infections), glomerulonephritis, obesity, diabetes, diabetes mellitus, Schmid metaphyseal chondrodysplasia, corneal endothelial dystrophies, posterior polymorphous corneal dystrophy (PPCD), Fuchs endothelial corneal dystrophy (FECD), atherosclerosis, scurvy, cancer, gastrointestinal stromal tumours, osteosarcoma, chondroblastoma, giant cell tumor, spondylometaphyseal dysplasia japanese type (SMD), lymphomas (Non-Hodgkin's lymphoma (NHL), follicular lymphomas, Burkitt's lymphoma, mantle cell lymphoma (MCL), multiple myeloma (MM), leukemia (chronic lymphocytic leukemia/small lymphocity lymphoma (CLL/SLL)), diffuse large cell B cell lymphoma (DLCL), B cell hyperplasia, Osteogenesis Imperfecta, Ehlers-Danlos syndrome, susceptibility to dissection of cervical arteries, aortic aneurysm, otospondylomegaepiphyseal dysplasia, hearing loss

(deafness), Weissenbacher-Zweymuller syndrome, bone or skeletal disease, late-onset retinal degeneration (L-ORD), age-related macular degeneration (AMD), blindness, arthritis, rheumatoid arthritis (RA), osteoarthritis, lyme arthritis, juvenile chronic arthritis, spondyloarthropathies, Systemic lupus erythematosus (SLE), Sjögren syndrome, demyelinating diseases of the central and peripheral nervous systems such as multiple sclerosis, idiopathic demyelinating polyneuropathy or Guillain-Barre syndrome, and chronic inflammatory demyelinating polyneuropathy, myasthenia gravis, bronchitis, emphysema, renal failure (glomerulonephritis, vasculitis, nephritis or pyrlonephritis), renal neoplasms, renal cell carcinomas, renal tumour, light chain neuropathy or amyloidosis, acute or chronic immune disease associated with organ transplantation, organ transplant rejection, graft-versus-host disease, Crohn's Disease, systemic sclerosis, idiopathic inflammatory myopathies, systemic vasculitis, sarcoidosis, autoimmune hemolytic anemia, autoimmune thrombocytopenia, thyroiditis, immune-mediated renal disease, hepatobiliary diseases such as infectious, autoimmune chronic active hepatitis, primary biliary cirrhosis, granulomatous hepatitis, and sclerosing cholangitis, inflammatory bowel disease, gluten-sensitive enteropathy, and Whipple's disease, autoimmune or immune-mediated skin diseases including bullous skin diseases, erythema multiforme and contact dermatitis, psoriasis, atopic dermatitis, food hypersensitivity and urticaria, immunologic diseases of the lung such as eosinophilic pneumonias, idiopathic pulmonary fibrosis and hypersensitivity pneumonitis, ulcerative colitis, inflammatory bowel disease, allergic diseases such as asthma, allergic rhinitis, sarcoidosis, female infertility, autoimmune thrombocytopenia, autoimmune thyroid disease, Hashimoto's disease, Sjogren's syndrome, ectodermal dysplasia, X-linked hypohidrotic ectodermal dysplasia (HED), inflammatory, ischemic or neoplastic diseases of the adrenal cortex, adrenal tumour, ganglioneuroblastoma, neuroblastoma, phaeochromocytomas, corstisol-producing adrenocortical adenomas, diseases linked to spinocerebellar degeneration, cerebellar diseases, olivopontocerebellar atrophy (OPCA) and/or Shy-Drager syndrome.

These molecules may also be used in the manufacture of a medicament for the treatment of such diseases. These molecules may also be used in contraception or for the treatment of reproductive disorders including infertility.

In a ninth aspect, the invention provides a method of diagnosing a disease in a patient, comprising assessing the level of expression of a natural gene encoding a polypeptide of the first aspect of the invention or the activity of a polypeptide of the first aspect of the

Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. In fact, blockage of the amino or carboxyl terminus in a polypeptide, or both, by a covalent modification is common in naturally-occurring and synthetic polypeptides and such modifications may be present in polypeptides of the present invention.

The modifications that occur in a polypeptide often will be a function of how the polypeptide is made. For polypeptides that are made recombinantly, the nature and extent of the modifications in large part will be determined by the post-translational modification capacity of the particular host cell and the modification signals that are present in the amino acid sequence of the polypeptide in question. For instance, glycosylation patterns vary between different types of host cell.

The polypeptides of the present invention can be prepared in any suitable manner. Such polypeptides include isolated naturally-occurring polypeptides (for example purified from cell culture), recombinantly-produced polypeptides (including fusion proteins), synthetically-produced polypeptides or polypeptides that are produced by a combination of these methods.

The functionally-equivalent polypeptides of the first aspect of the invention may be polypeptides that are homologous to the INSP163 polypeptides. Two polypeptides are said to be "homologous", as the term is used herein, if the sequence of one of the polypeptides has a high enough degree of identity or similarity to the sequence of the other polypeptide. "Identity" indicates that at any particular position in the aligned sequences, the amino acid residue is identical between the sequences. "Similarity" indicates that, at any particular position in the aligned sequences, the amino acid residue is of a similar type between the sequences. Degrees of identity and similarity can be readily calculated (Computational Molecular Biology, Lesk, A.M., ed., Oxford University Press, New York, 1988; Biocomputing. Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part 1, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). Percentage identity, as referred to herein, is as determined using BLAST version 2.1.3 using the default parameters specified by the NCBI (the National Center for Biotechnology Information; http://www.ncbi.nlm.nih.gov/) [Blosum 62 matrix; gap open penalty=11 and gap extension

10% and above.

Polypeptides may be divided into fragments and similarly fragments of functional equivalents may exist. Such fragments are identified by being members of the same protein family as the full-length polypeptide, or having an antigenic determinant in common with the full-length polypeptides.

As used herein, the term "fragment" refers to a polypeptide having an amino acid sequence that is the same as part, but not all, of the amino acid sequence of a polypeptide or one of the functional equivalents of that polypeptide. The fragments should comprise at least n consecutive amino acids from the sequence and, depending on the particular sequence, n preferably is 7 or more (for example, 8, 10, 12, 14, 16, 18, 20 or more). Small fragments may form an antigenic determinant.

Fragments of full length polypeptides may consist of combinations of 1, 2, 3, 4, 5, 6, 7 or all 8 neighbouring exon sequences in the polypeptide sequences, respectively. For example, such combinations include exons 1 and 2, exons 2 and 3 or exons 1, 2 and 3, and so on. Such fragments are included in the present invention.

Such fragments may be "free-standing", *i.e.* not part of or fused to other amino acids or polypeptides, or they may be comprised within a larger polypeptide of which they form a part or region. When comprised within a larger polypeptide, the fragment of the invention most preferably forms a single continuous region. For instance, certain preferred embodiments relate to a fragment having a pre- and/or pro- polypeptide region fused to the amino terminus of the fragment and/or an additional region fused to the carboxyl terminus of the fragment. However, several fragments may be comprised within a single larger polypeptide.

The polypeptides of the present invention or their immunogenic fragments (comprising at least one antigenic determinant) can be used to generate ligands, such as polyclonal or monoclonal antibodies, that are immunospecific for the polypeptides. Such antibodies may be employed to isolate or to identify clones expressing the polypeptides of the invention or to purify the polypeptides by affinity chromatography. The antibodies may also be employed as diagnostic or therapeutic aids, amongst other applications, as will be apparent to the skilled reader.

The term "immunospecific" means that the antibodies have substantially greater affinity for the polypeptides of the invention than their affinity for other related polypeptides in the

constant regions (see, for example, Liu *et al.*, Proc. Natl. Acad. Sci. USA, 84, 3439 (1987)), may also be of use.

The antibody may be modified to make it less immunogenic in an individual, for example by humanisation (see Jones *et al.*, Nature, 321, 522 (1986); Verhoeyen *et al.*, Science, 239, 1534 (1988); Kabat *et al.*, J. Immunol., 147, 1709 (1991); Queen *et al.*, Proc. Natl. Acad. Sci. USA, 86, 10029 (1989); Gorman *et al.*, Proc. Natl Acad. Sci. USA, 88, 34181 (1991); and Hodgson *et al.*, Bio/Technology, 9, 421 (1991)). The term "humanised antibody", as used herein, refers to antibody molecules in which the CDR amino acids and selected other amino acids in the variable domains of the heavy and/or light chains of a non-human donor antibody have been substituted in place of the equivalent amino acids in a human antibody. The humanised antibody thus closely resembles a human antibody but has the binding ability of the donor antibody.

In a further alternative, the antibody may be a "bispecific" antibody, that is, an antibody having two different antigen binding domains, each domain being directed against a different epitope.

Phage display technology may be utilised to select genes which encode antibodies with binding activities towards the polypeptides of the invention either from repertoires of PCR amplified V-genes of lymphocytes from humans screened for possessing the relevant antibodies, or from naive libraries (McCafferty, J. *et al.*, (1990), Nature 348, 552-554; Marks, J. *et al.*, (1992) Biotechnology 10, 779-783). The affinity of these antibodies can also be improved by chain shuffling (Clackson, T. *et al.*, (1991) Nature 352, 624-628).

Antibodies generated by the above techniques, whether polyclonal or monoclonal, have additional utility in that they may be employed as reagents in immunoassays, radioimmunoassays (RIA) or enzyme-linked immunosorbent assays (ELISA). In these applications, the antibodies can be labelled with an analytically-detectable reagent such as a radioisotope, a fluorescent molecule or an enzyme.

Preferred nucleic acid molecules of the second and third aspects of the invention are those which encode a polypeptide sequence as recited in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34 and/or SEQ ID NO: 36 and functionally equivalent polypeptides. These nucleic acid molecules may be used in the

methods and applications described herein. The nucleic acid molecules of the invention preferably comprise at least n consecutive nucleotides from the sequences disclosed herein where, depending on the particular sequence, n is 10 or more (for example, 12, 14, 15, 18, 20, 25, 30, 35, 40 or more).

The nucleic acid molecules of the invention also include sequences that are complementary to nucleic acid molecules described above (for example, for antisense or probing purposes).

Nucleic acid molecules of the present invention may be in the form of RNA, such as mRNA, or in the form of DNA, including, for instance cDNA, synthetic DNA or genomic DNA. Such nucleic acid molecules may be obtained by cloning, by chemical synthetic techniques or by a combination thereof. The nucleic acid molecules can be prepared, for example, by chemical synthesis using techniques such as solid phase phosphoramidite chemical synthesis, from genomic or cDNA libraries or by separation from an organism. RNA molecules may generally be generated by the *in vitro* or *in vivo* transcription of DNA sequences.

The nucleic acid molecules may be double-stranded or single-stranded. Single-stranded DNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

The term "nucleic acid molecule" also includes analogues of DNA and RNA, such as those containing modified backbones, and peptide nucleic acids (PNA). The term "PNA", as used herein, refers to an antisense molecule or an anti-gene agent which comprises an oligonucleotide of at least five nucleotides in length linked to a peptide backbone of amino acid residues, which preferably ends in lysine. The terminal lysine confers solubility to the composition. PNAs may be pegylated to extend their lifespan in a cell, where they preferentially bind complementary single stranded DNA and RNA and stop transcript elongation (Nielsen, P.E. *et al.* (1993) Anticancer Drug Des. 8:53-63).

A nucleic acid molecule which encodes a polypeptide of this invention may be identical to the coding sequence of one or more of the nucleic acid molecules disclosed herein.

These molecules also may have a different sequence which, as a result of the degeneracy of the genetic code, encodes a polypeptide SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34 or SEQ ID NO: 36 . Such nucleic acid

molecules may include, but are not limited to, the coding sequence for the mature polypeptide by itself; the coding sequence for the mature polypeptide and additional coding sequences, such as those encoding a leader or secretory sequence, such as a pro-, pre- or prepro- polypeptide sequence; the coding sequence of the mature polypeptide, with or without the aforementioned additional coding sequences, together with further additional, non-coding sequences, including non-coding 5' and 3' sequences, such as the transcribed, non-translated sequences that play a role in transcription (including termination signals), ribosome binding and mRNA stability. The nucleic acid molecules may also include additional sequences which encode additional amino acids, such as those which provide additional functionalities.

The nucleic acid molecules of the second and third aspects of the invention may also encode the functional equivalents of the polypeptides of the first aspect of the invention. Such a nucleic acid molecule may be a naturally-occurring variant such as a naturally-occurring allelic variant, or the molecule may be a variant that is not known to occur naturally. Such non-naturally occurring variants of the nucleic acid molecule may be made by mutagenesis techniques, including those applied to nucleic acid molecules, cells or organisms.

Among variants in this regard are variants that differ from the aforementioned nucleic acid molecules by nucleotide substitutions, deletions or insertions. The substitutions, deletions or insertions may involve one or more nucleotides. The variants may be altered in coding or non-coding regions or both. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or insertions.

The nucleic acid molecules of the invention can also be engineered, using methods generally known in the art, for a variety of reasons, including modifying the cloning, processing, and/or expression of the gene product (the polypeptide). DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides are included as techniques which may be used to engineer the nucleotide sequences. Site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, introduce mutations and so forth.

Nucleic acid molecules which encode a polypeptide of the first aspect of the invention may be ligated to a heterologous sequence so that the combined nucleic acid molecule encodes a

"Stringency" refers to conditions in a hybridization reaction that favour the association of very similar molecules over association of molecules that differ. High stringency hybridisation conditions are defined as overnight incubation at 42°C in a solution comprising 50% formamide, 5XSSC (150mM NaCl, 15mM trisodium citrate), 50mM sodium phosphate (pH7.6), 5x Denhardts solution, 10% dextran sulphate, and 20 microgram/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1X SSC at approximately 65°C. Low stringency conditions involve the hybridisation reaction being carried out at 35°C (see Sambrook *et al.* [*supra*]). Preferably, the conditions used for hybridization are those of high stringency.

Preferred embodiments of this aspect of the invention are nucleic acid molecules that are at least 70% identical over their entire length to a nucleic acid molecule encoding the INSP163 polypeptides and nucleic acid molecules that are substantially complementary to such nucleic acid molecules. Preferably, a nucleic acid molecule according to this aspect of the invention comprises a region that is at least 80% identical over its entire length to such coding sequences, or is a nucleic acid molecule that is complementary thereto. In this regard, nucleic acid molecules at least 90%, preferably at least 95%, more preferably at least 98%, 99% or more identical over their entire length to the same are particularly preferred. Preferred embodiments in this respect are nucleic acid molecules that encode polypeptides which retain substantially the same biological function or activity as the INSP163 polypeptides.

The invention also provides a process for detecting a nucleic acid molecule of the invention, comprising the steps of: (a) contacting a nucleic probe according to the invention with a biological sample under hybridizing conditions to form duplexes; and (b) detecting any such duplexes that are formed.

As discussed additionally below in connection with assays that may be utilised according to the invention, a nucleic acid molecule as described above may be used as a hybridization probe for RNA, cDNA or genomic DNA, in order to isolate full-length cDNAs and genomic clones encoding the INSP163 polypeptides and to isolate cDNA and genomic clones of homologous or orthologous genes that have a high sequence similarity to the gene encoding this polypeptide.

In this regard, the following techniques, among others known in the art, may be utilised and are discussed below for purposes of illustration. Methods for DNA sequencing and analysis

method which may be employed is based on the method of Rapid Amplification of cDNA Ends (RACE; see, for example, Frohman *et al.*, PNAS USA 85, 8998-9002, 1988). Recent modifications of this technique, exemplified by the Marathon™ technology (Clontech Laboratories Inc.), for example, have significantly simplified the search for longer cDNAs. A slightly different technique, termed "restriction-site" PCR, uses universal primers to retrieve unknown nucleic acid sequence adjacent a known locus (Sarkar, G. (1993) PCR Methods Applic. 2:318-322). Inverse PCR may also be used to amplify or to extend sequences using divergent primers based on a known region (Triglia, T. *et al.* (1988) Nucleic Acids Res. 16:8186). Another method which may be used is capture PCR which involves PCR amplification of DNA fragments adjacent a known sequence in human and yeast artificial chromosome DNA (Lagerstrom, M. *et al.* (1991) PCR Methods Applic., 1, 111-119). Another method which may be used to retrieve unknown sequences is that of Parker, J.D. *et al.* (1991); Nucleic Acids Res. 19:3055-3060). Additionally, one may use PCR, nested primers, and PromoterFinder™ libraries to walk genomic DNA (Clontech, Palo Alto, CA). This process avoids the need to screen libraries and is useful in finding intron/exon junctions.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. Also, random-primed libraries are preferable, in that they will contain more sequences that contain the 5' regions of genes. Use of a randomly primed library may be especially preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into 5' non-transcribed regulatory regions.

In one embodiment of the invention, the nucleic acid molecules of the present invention may be used for chromosome localisation. In this technique, a nucleic acid molecule is specifically targeted to, and can hybridize with, a particular location on an individual human chromosome. The mapping of relevant sequences to chromosomes according to the present invention is an important step in the confirmatory correlation of those sequences with the gene-associated disease. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found in, for example, V. McKusick, Mendelian Inheritance in Man (available on-line through Johns Hopkins University Welch Medical Library). The relationships between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of

physically adjacent genes). This provides valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the disease or syndrome has been crudely localised by genetic linkage to a particular genomic region, any sequences mapping to that area may represent associated or regulatory genes for further investigation. The nucleic acid molecule may also be used to detect differences in the chromosomal location due to translocation, inversion, *etc.* among normal, carrier, or affected individuals.

The nucleic acid molecules of the present invention are also valuable for tissue localisation. Such techniques allow the determination of expression patterns of the polypeptide in tissues by detection of the mRNAs that encode them. These techniques include *in situ* hybridization techniques and nucleotide amplification techniques, such as PCR. Results from these studies provide an indication of the normal functions of the polypeptide in the organism. In addition, comparative studies of the normal expression pattern of mRNAs with that of mRNAs encoded by a mutant gene provide valuable insights into the role of mutant polypeptides in disease. Such inappropriate expression may be of a temporal, spatial or quantitative nature.

Gene silencing approaches may also be undertaken to down-regulate endogenous expression of a gene encoding a polypeptide of the invention. RNA interference (RNAi) (Elbashir, SM *et al.*, Nature 2001, 411, 494-498) is one method of sequence specific post-transcriptional gene silencing that may be employed. Short dsRNA oligonucleotides are synthesised *in vitro* and introduced into a cell. The sequence specific binding of these dsRNA oligonucleotides triggers the degradation of target mRNA, reducing or ablating target protein expression.

Efficacy of the gene silencing approaches assessed above may be assessed through the measurement of polypeptide expression (for example, by Western blotting), and at the RNA level using TaqMan-based methodologies.

The vectors of the present invention comprise nucleic acid molecules of the invention and may be cloning or expression vectors. The host cells of the invention, which may be transformed, transfected or transduced with the vectors of the invention may be prokaryotic or eukaryotic.

The polypeptides of the invention may be prepared in recombinant form by expression of their encoding nucleic acid molecules in vectors contained within a host cell. Such

containing the appropriate sequences can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding a polypeptide of the invention under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

Alternatively, host cells that contain a nucleic acid sequence encoding a polypeptide of the invention and which express said polypeptide may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassays, for example, fluorescence activated cell sorting (FACS) or immunoassay techniques (such as the enzyme-linked immunosorbent assay [ELISA] and radioimmunoassay [RIA]), that include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein (see Hampton, R. *et al.* (1990) Serological Methods, a Laboratory Manual, APS Press, St Paul, MN) and Maddox, D.E. *et al.* (1983) J. Exp. Med, 158, 1211-1216).

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labelled hybridization or PCR probes for detecting sequences related to nucleic acid molecules encoding polypeptides of the present invention include oligolabelling, nick translation, end-labelling or PCR amplification using a labelled polynucleotide. Alternatively, the sequences encoding the polypeptide of the invention may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesise RNA probes *in vitro* by addition of an appropriate RNA polymerase such as T7, T3 or SP6 and labelled nucleotides. These procedures may be conducted using a variety of commercially available kits (Pharmacia & Upjohn, (Kalamazoo, MI); Promega (Madison WI); and U.S. Biochemical Corp. (Cleveland, OH)).

Suitable reporter molecules or labels, which may be used for ease of detection, include radionucleides, enzymes and fluorescent, chemiluminescent or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Nucleic acid molecules according to the present invention may also be used to create transgenic animals, particularly rodent animals. Such transgenic animals form a further aspect of the present invention. This may be done locally by modification of somatic cells,

screening assays may be used, in which neutralising antibodies that are capable of binding the polypeptide specifically compete with a test compound for binding. In this manner, the antibodies can be used to detect the presence of any test compound that possesses specific binding affinity for the polypeptide.

Assays may also be designed to detect the effect of added test compounds on the production of mRNA encoding the polypeptide in cells. For example, an ELISA may be constructed that measures secreted or cell-associated levels of polypeptide using monoclonal or polyclonal antibodies by standard methods known in the art, and this can be used to search for compounds that may inhibit or enhance the production of the polypeptide from suitably manipulated cells or tissues. The formation of binding complexes between the polypeptide and the compound being tested may then be measured.

Assay methods that are also included within the terms of the present invention are those that involve the use of the genes and polypeptides of the invention in overexpression or ablation assays. Such assays involve the manipulation of levels of these genes/polypeptides in cells and assessment of the impact of this manipulation event on the physiology of the manipulated cells. For example, such experiments reveal details of signaling and metabolic pathways in which the particular genes/polypeptides are implicated, generate information regarding the identities of polypeptides with which the studied polypeptides interact and provide clues as to methods by which related genes and proteins are regulated.

Another technique for drug screening which may be used provides for high throughput screening of compounds having suitable binding affinity to the polypeptide of interest (see International patent application WO84/03564). In this method, large numbers of different small test compounds are synthesised on a solid substrate, which may then be reacted with the polypeptide of the invention and washed. One way of immobilising the polypeptide is to use non-neutralising antibodies. Bound polypeptide may then be detected using methods that are well known in the art. Purified polypeptide can also be coated directly onto plates for use in the aforementioned drug screening techniques.

The polypeptide of the invention may be used to identify membrane-bound or soluble receptors, through standard receptor binding techniques that are known in the art, such as ligand binding and crosslinking assays in which the polypeptide is labelled with a radioactive isotope, is chemically modified, or is fused to a peptide sequence that facilitates its detection or purification, and incubated with a source of the putative receptor (for

(proprotein convertase subtilisin/kexin type 7) is a closely related to PACE and PACE4. This calcium-dependent serine endoprotease is concentrated in the trans-Golgi network, associated with the membranes, and is not secreted. It can process proalbumin. PC7 and furin are also thought to be one of the proteases responsible for the activation of HIV envelope glycoproteins gp160 and gp140.

N-Arg dibasic convertase (NDR) is a metalloendopeptidase primarily cloned from rat brain cortex and testis that cleaves peptide substrates on the N terminus of Arg residues in dibasic stretches. It hydrolyses polypeptides, preferably at -Xaa-+-Arg-Lys-, and less commonly at -Arg-+-Arg-Xaa-, in which Xaa is not Arg or Lys. It has been proved that it can cleave alpha-neoendorphin, ANF, dynorphin, preproneurotensin and somatostatin. Also there is an evidence for extracellular localization of active NDR.

Table 1

Protein convertase	**Motif**	**Residues**
N-Arg dibasic convertase (nardilysine) cleavage site (Xaa-|-Arg-Lys or Arg-|-Arg-Xaa)	RRP LRK RRF RRV	80-82 87-89 131-133 162-164
NEC1/NEC2 cleavage site (Lys-Arg-|-Xaa)	KRC KRT	89-91 166-168
Proprotein convertase 7 (PC7, PCSK7) cleavage site (Arg-Xaa-Xaa-Xaa-[Arg/Lys]-Arg-|-Xaa)	RRVDKRT	162-168

Example 4: Cloning of INSP163

Preparation of human cDNA templates

First strand cDNA was prepared from a variety of human tissue total RNA samples (Clontech, Stratagene, Ambion, Biochain Institute and in-house preparations) using Superscript II or SuperScript III RNase H⁻ Reverse Transcriptase (Invitrogen) according to the manufacturer's protocol.

For SuperScript II: Oligo $(dT)_{15}$ primer (1µl at 500µg/ml) (Promega), 2µg human total RNA, 1µl 10mM dNTP mix (10mM each of dATP, dGTP, dCTP and dTTP at neutral pH) and sterile distilled water to a final volume of 12µl were combined in a 1.5ml Eppendorf tube, heated to 65°C for 5 min and chilled on ice. The contents were collected by brief centrifugation and 4µl of 5X First-Strand Buffer, 2µl 0.1 M DTT, and 1µl RnaseOUT™ Recombinant Ribonuclease Inhibitor (40 units/µl, Invitrogen) were added. The contents of the tube were mixed gently and incubated at 42°C for 2 min, then 1µl (200 units) of SuperScript II™ enzyme was added and mixed gently by pipetting. The mixture was incubated at 42°C for 50 min and then inactivated by heating at 70°C for 15 min. To remove RNA complementary to the cDNA, 1µl (2 units) of *E. coli* RNase H (Invitrogen) was added and the reaction mixture incubated at 37°C for 20 min.

For SuperScript III: 1µl Oligo$(dT)_{20}$ primer (50µM, Invitrogen), 2µg human total RNA, 1µl 10mM dNTP mix (10mM each of dATP, dGTP, dCTP and dTTP at neutral pH) and sterile distilled water to a final volume of 10µl were combined in a 1.5ml Eppendorf tube, heated to 65°C for 5 min and then chilled on ice. For each RT reaction a cDNA synthesis mix was prepared as follows: 2µl 10X RT buffer, 4µl 25mM $MgCl_2$, 2µl 0.1M DTT, 1µl RNaseOUT™ (40U/µl) and 1µl SuperScript III™ RT enzyme were combined in a separate tube and then 10µl of this mix added to the tube containing the RNA/primer mixture. The contents of the tube were mixed gently, collected by brief centrifugation, and incubated at 50°C for 50 min. The reaction was terminated by incubating at 80°C for 5 min and the reaction mixture then chilled on ice and collected by brief centrifugation. To remove RNA complementary to the cDNA, 1µl (2 units) of *E. coli* RNase H (Invitrogen) was added and the reaction mixture incubated at 37°C for 20 min.

The final 21µl reaction mix was diluted by adding 179µl sterile water to give a total volume of 200µl. This represented approximately 20ng/µl of each individual cDNA template.

Gene specific cloning primers for PCR

A pair of PCR primers having a length of between 18 and 30 bases were designed to amplify the full length of the INSP163 predicted cds using Primer Designer Software (Scientific & Educational Software, PO Box 72045, Durham, NC 27722-2045, USA). PCR primers were optimized to have a Tm close to 55 ± 10°C and a GC content of 40-60%.

Primers were selected which had high selectivity for the target sequence (INSP163) with little or no none specific priming.

PCR amplification of INSP163 from human cDNA templates

Gene-specific cloning primers (INSP163-CP1 and INSP163-CP2, Figure 2, Table 2) were designed to amplify a cDNA fragment of 929 bp covering the full length of the INSP163 cds. Interrogation of public EST sequence databases with the INSP163 prediction suggested that the sequence might be expressed in cDNA templates derived from normal kidney, Crohn's disease kidney and cancerous tissues. The primer pair was therefore used with individual cDNA samples from these sources as PCR templates. PCR was performed in a final volume of 50µl containing 1X Platinum® *Taq* High Fidelity (HiFi) buffer, 2mM $MgSO_4$, 200µM dNTPs, 0.2µM of each cloning primer, 1 unit of Platinum® *Taq* DNA Polymerase High Fidelity (HiFi) (Invitrogen), approximately 20ng of individual cDNA template, and 0X, 1X or 2X PCR_x Enhancer solution (Invitrogen). Cycling was performed using an MJ Research DNA Engine, programmed as follows: 94°C, 2 min; 40 cycles of 94°C, 30 sec, 66°C, 30 sec, and 68°C, 1 min; followed by 1 cycle at 68°C for 8 min and a holding cycle at 4°C.

30µl of each amplification product was visualized on a 0.8% agarose gel in 1 X TAE buffer (Invitrogen). Products of the expected molecular weight were purified from the gel using the MinElute DNA Purification System (Qiagen), eluted in 10µl of EB buffer (10mM Tris.Cl, pH 8.5) and subcloned directly.

Subcloning of PCR Products

The PCR products were subcloned into the topoisomerase I modified cloning vector (pCR4-TOPO) using the TA cloning kit purchased from the Invitrogen Corporation using the conditions specified by the manufacturer. Briefly, 4µl of gel purified PCR product was incubated for 15 min at room temperature with 1µl of TOPO vector and 1µl salt solution. The reaction mixture was then transformed into *E. coli* strain TOP10 (Invitrogen) as follows: a 50µl aliquot of One Shot TOP10 cells was thawed on ice and 2µl of TOPO reaction was added. The mixture was incubated for 15 min on ice and then heat shocked by incubation at 42°C for exactly 30 s. Samples were returned to ice and 250µl of warm (room

Plasmid mini-prep DNA was prepared from 5ml cultures from 6 of the resultant colonies subcloned in each vector using a Qiaprep BioRobot 8000 system (Qiagen). Plasmid DNA (200-500ng) in the pEAK12d vector was subjected to DNA sequencing with pEAK12F and pEAK12R primers as described above. Plasmid DNA (200-500ng) in the pDEST12.2 vector was subjected to DNA sequencing with 21M13 and M13Rev primers as described above. Primer sequences are shown in Table 2.

CsCl gradient purified maxi-prep DNA was prepared from a 500ml culture of the sequence verified clone (pEAK12d_INSP163-6HIS) using the method described by Sambrook J. *et al.*, 1989 (in Molecular Cloning, a Laboratory Manual, 2nd edition, Cold Spring Harbor Laboratory Press). Plasmid DNA was resuspended at a concentration of 1μg/μl in sterile water (or 10mM Tris-HCl pH 8.5) and stored at -20°C.

Endotoxin-free maxi-prep DNA was prepared from a 500ml culture of the sequence verified clone (pDEST12.2_INSP163-6HIS) using the EndoFree Plasmid Mega kit (Qiagen) according to the manufacturer's instructions. Purified plasmid DNA was resuspended in endotoxin free TE buffer at a final concentration of at least 3 μg/μl and stored at -20°C.

Example 6: Expression and purification of INSP163

Further experiments may now be performed to determine the tissue distribution and expression levels of the INSP163 polypeptide *in vivo*, on the basis of the nucleotide and amino acid sequence disclosed herein.

The presence of the transcripts for INSP163 may be investigated by PCR of cDNA from different human tissues. The INSP163 transcripts may be present at very low levels in the samples tested. Therefore, extreme care is needed in the design of experiments to establish the presence of a transcript in various human tissues as a small amount of genomic contamination in the RNA preparation will provide a false positive result. Thus, all RNA should be treated with DNAse prior to use for reverse transcription. In addition, for each tissue a control reaction may be set up in which reverse transcription was not undertaken (a -ve RT control).

For example, 1μg of total RNA from each tissue may be used to generate cDNA using Multiscript reverse transcriptase (ABI) and random hexamer primers. For each tissue, a

Purification process

The culture medium sample containing the recombinant protein with a C-terminal 6His tag is diluted with cold buffer A (50mM NaH_2PO_4; 600mM NaCl; 8.7 % (w/v) glycerol, pH 7.5). The sample is filtered then through a sterile filter (Millipore) and kept at 4°C in a sterile square media bottle (Nalgene).

The purification is performed at 4°C on the VISION workstation (Applied Biosystems) connected to an automatic sample loader (Labomatic). The purification procedure is composed of two sequential steps, metal affinity chromatography on a Poros 20 MC (Applied Biosystems) column charged with Ni ions (4.6 x 50 mm, 0.83ml), followed by gel filtration on a Sephadex G-25 medium (Amersham Pharmacia) column (1,0 x 10cm).

For the first chromatography step the metal affinity column is regenerated with 30 column volumes of EDTA solution (100mM EDTA; 1M NaCl; pH 8.0), recharged with Ni ions through washing with 15 column volumes of a 100mM $NiSO_4$ solution, washed with 10 column volumes of buffer A, followed by 7 column volumes of buffer B (50mM NaH_2PO_4; 600mM NaCl; 8.7 % (w/v) glycerol, 400mM; imidazole, pH 7.5), and finally equilibrated with 15 column volumes of buffer A containing 15mM imidazole. The sample is transferred, by the Labomatic sample loader, into a 200ml sample loop and subsequently charged onto the Ni metal affinity column at a flow rate of 10ml/min. The column is washed with 12 column volumes of buffer A, followed by 28 column volumes of buffer A containing 20mM imidazole. During the 20mM imidazole wash loosely attached contaminating proteins are eluted from the column. The recombinant His-tagged protein is finally eluted with 10 column volumes of buffer B at a flow rate of 2ml/min, and the eluted protein is collected.

For the second chromatography step, the Sephadex G-25 gel-filtration column is regenerated with 2ml of buffer D (1.137M NaCl; 2.7mM KCl; 1.5mM KH_2PO_4; 8mM Na_2HPO_4; pH 7.2), and subsequently equilibrated with 4 column volumes of buffer C (137mM NaCl; 2.7mM KCl; 1.5mM KH_2PO_4; 8mM Na_2HPO_4; 20% (w/v) glycerol; pH 7.4). The peak fraction eluted from the Ni-column is automatically loaded onto the Sephadex G-25 column through the integrated sample loader on the VISION and the protein is eluted with buffer C at a flow rate of 2 ml/min. The fraction was filtered through a sterile centrifugation filter (Millipore), frozen and stored at –80°C. An aliquot of the sample is analyzed on SDS-PAGE (4-12% NuPAGE gel; Novex) Western blot with anti-

His antibodies. The NuPAGE gel may be stained in a 0.1 % Coomassie blue R250 staining solution (30% methanol, 10% acetic acid) at room temperature for 1h and subsequently destained in 20% methanol, 7.5% acetic acid until the background is clear and the protein bands clearly visible.

Following the electrophoresis the proteins are electrotransferred from the gel to a nitrocellulose membrane. The membrane is blocked with 5% milk powder in buffer E (137mM NaCl; 2.7mM KCl; 1.5mM KH_2PO_4; 8mM Na_2HPO_4; 0.1 % Tween 20, pH 7.4) for 1h at room temperature, and subsequently incubated with a mixture of 2 rabbit polyclonal anti-His antibodies (G-18 and H-15, 0.2µg/ml each; Santa Cruz) in 2.5% milk powder in buffer E overnight at 4°C. After a further 1 hour incubation at room temperature, the membrane is washed with buffer E (3 x 10min), and then incubated with a secondary HRP-conjugated anti-rabbit antibody (DAKO, HRP 0399) diluted 1/3000 in buffer E containing 2.5% milk powder for 2 hours at room temperature. After washing with buffer E (3 x 10 minutes), the membrane is developed with the ECL kit (Amersham Pharmacia) for 1 min. The membrane is subsequently exposed to a Hyperfilm (Amersham Pharmacia), the film developed and the western blot image visually analysed.

For samples that showed detectable protein bands by Coomassie staining, the protein concentration may be determined using the BCA protein assay kit (Pierce) with bovine serum albumin as standard.

Furthermore, overexpression or knock-down of the expression of the polypeptides in cell lines may be used to determine the effect on transcriptional activation of the host cell genome. Dimerisation partners, co-activators and co-repressors of the INSP163 polypeptide may be identified by immunoprecipitation combined with Western blotting and immunoprecipitation combined with mass spectroscopy.

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,700,724

APPLICATION NO.: 10/573,936

DATED : April 20, 2010

INVENTORS : Stephen Noel Fitzgerald, Richard Joseph Fagan, Christine Power, Melanie Yorke, Jadwiga Bienkowska

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 2,
Line 32, "MRNA" should read --mRNA--.

Column 5,
Line 47, "β-amayloid" should read --β-amyloid--.

Column 10,
Lines 4-5, "osteoarritis" should read --osteoarthritis--.

Column 13,
Line 36, "comeal dystrophy" should read --corneal dystrophy--.
Line 43, "diffuise large cell" should read --diffuse large cell--.
Line 50, "athritis" should read --arthritis--.

Column 18,
Line 17, "used herin" should read --used herein--.

Column 19,
Line 43, "singie" should read --single--.

Column 20,
Line 46, "Jones et aL," should read --Jones et al.,--.
Line 48, "Queen et aL," should read --Queen et al.,--.

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Column 21,
Line 31, "MRNA" should read --mRNA--.
Line 32, "CDNA" should read --cDNA--.

Column 22,
Line 11, "MRNA" should read --mRNA--.

Column 23,
Line 33, "15 nM" should read --15mM--.

Column 25,
Line 34, "Johns Hopldns" should read --Johns Hopkins--.

Column 26,
Line 2, "MRNA" should read --mRNA--.

Column 29,
Line 29, "MRNA" should read --mRNA--.

Column 32,
Line 34, "MRNA" should read --mRNA--.

Column 44,
Line 22, "or SuperScript II" should read --or SuperScript III--.
Line 46, "4 μl 25 nM" should read --4 μl 25 mM--.

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It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 45,
Line 17, "2 nM" should read --2mM--.

Column 48,
Line 62, "(or 10 nM" should read --(or 10mM--.

Column 50,
Line 11, "600 nM" should read --600mM--.
Line 32, "15 nM" should read --15mM--.
Line 45, "2.7 nM KCl; 1.5 nM KH_2PO_4; 8 nM" should read
--2.7mM KCl; 1.5mM KH_2PO_4; 8mM--.
Lines 47-48, "137 nM NaCl; 2.7 nM KCl; 1.5 nM" should read
--137mM NaCl; 2.7mM KCl; 1.5mM--.
Lines 63-64, "137 nM NaCl; 2.7 nM KCl; 1.5 nM KH_2PO_4; 8 nM" should read
--137mM NaCl; 2.7mM KCl; 1.5mM KH_2PO_4; 8mM--.

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